

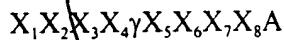
CLAIMS

We claim:

- Sue B* 1. In a polyamide which specifically binds to base pairs in the minor groove of a DNA molecule, the improvement comprising a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or the major groove of a DNA molecule.
- 5
- 10 2. A polyamide of claim 1 wherein the rigid group comprises a first and a second amino acid; said first amino acid being selected from the group consisting of arginine, proline, lysine, hydroxyproline and a derivative thereof; and said second amino acid being selected from the group consisting of proline, glycine, serine, threonine, leucine, isoleucine, valine, alanine, hydroxyproline and a derivative thereof.
- 15 3. A polyamide of claim 2 wherein said first amino acid is arginine and said second amino acid is proline.
- 20 4. A polyamide of claim 1 wherein the positively charged group comprises a synthetic or naturally occurring amino acid having a net positive charge.
5. A polyamide of claim 1 wherein said positively charged group is selected from the group consisting of a primary amino group, secondary amino group, tertiary amino group, quartenary amino group, guanidinium group, and an amidinium group.
- 25 6. A polyamide of claim 1 wherein said positively charged group is selected from the group consisting of arginine, lysine, histidine and a derivative thereof.
- Sue B2* 7. A polyamide of claim 1 wherein said positively charged group is arginine.

8. A polyamide of claim 1 wherein the positive patch comprises the amino acid sequence Arg-Pro-Arg.
9. A polyamide of claim 1 wherein the polyamide has three or four carboxamide binding pairs.  
5
10. A polyamide of claim 1 wherein the polyamide comprises an (R)-2,4-diaminobutyric acid hairpin turn that facilitates specific binding to base pairs in the minor groove of a DNA molecule.  
10
11. A polyamide of claim 10 wherein the R-2-amino group is derivatized to form an acid amide.
12. A polyamide of claim 1 having the formula:  
*Spec 82*  
$$X_1 X_2 X_3 \gamma X_4 X_5 X_6 A$$
wherein  $\gamma$  is  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CONH}-$  hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;  
20  
 $X_1/X_6$ ,  $X_2/X_5$ , and  $X_3/X_4$  represent three carboxamide binding pairs which bind DNA base pairs and are selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound; and  
25  
A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.
13. A polyamide of claim 12 wherein the positive patch comprises the amino acid sequence Arg-Pro-Arg.

*Sub B* 14 A polyamide of claim 1 having the formula:



wherein  $\gamma$  is -NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CONH- hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

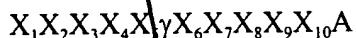
10  $X_1/X_8$ ,  $X_2/X_7$ ,  $X_3/X_6$ , and  $X_4/X_5$  represent four carboxamide binding pairs which bind DNA base pairs and are selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound; and

A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

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15. A polyamide of claim 14 wherein the positive patch comprises the amino acid sequence Arg-Pro-Arg.

*Sub B* 16. A polyamide of claim 1 having the formula:



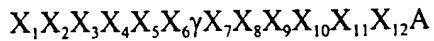
wherein  $\gamma$  is -NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CONH- hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

25  $X_1/X_{10}$ ,  $X_2/X_9$ ,  $X_3/X_8$ ,  $X_4/X_7$ ,  $X_5/X_6$  represent five carboxamide binding pairs which bind DNA base pairs and are selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound; and

30 A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

17. A polyamide of claim 16 wherein the positive patch comprises the amino acid sequence Arg-Pro-Arg.

*Sub B6* 18. A polyamide of claim 1 having the formula:



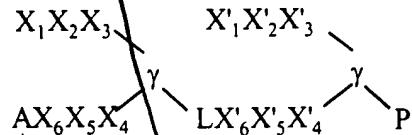
wherein  $\gamma$  is  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CONH}-$  hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

10  $X_1/X_{12}$ ,  $X_2/X_{11}$ ,  $X_3/X_{10}$ ,  $X_4/X_9$ ,  $X_5/X_8$ ,  $X_6/X_7$  represent three or four carboxamide binding pairs which bind DNA base pairs and are selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound; and

15 A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

19. A polyamide of claim 18 wherein the positive patch comprises the amino acid sequence Arg-Pro-Arg.

*Sub B70* 20. A tandem-linked polyamide having the formula:



wherein  $\gamma$  is  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CONH}-$  hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

25  $X_1/X_6$ ,  $X_2/X_5$ ,  $X_3/X_4$ ,  $X'_1/X'_6$ ,  $X'_2/X'_5$ ,  $X'_3/X'_4$  represent carboxamide binding pairs which bind DNA base pairs selected from the group consisting of Hp/Py, Py/Hp,

Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound;

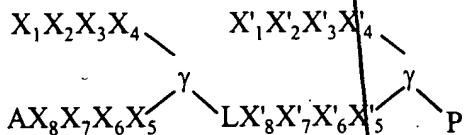
L represents an amino acid linking group selected from the group consisting of  $\beta$ -alanine and 5-aminovaleric acid ( $\delta$ );

5 P represents a polyamide selected from the group consisting of

$X_1X_2X_3\gamma X_4X_5X_6$ ,  $X_1X_2X_3X_4\gamma X_5X_6X_7X_8$ ,  $X_1X_2X_3X_4X_5\gamma X_6X_7X_8X_9X_{10}$ , and  $X_1X_2X_3X_4X_5X_6\gamma X_7X_8X_9X_{10}X_{11}X_{12}$  where  $X_1-X_{12}$  are independently selected from the group consisting of  $\beta$ -alanine, pyrrole, hydroxypyrrrole and imidazole; and

A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

10 21. A tandem-linked polyamide having the formula:



wherein  $\gamma$  is  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CONH}-$  hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

15  $X_1/X_8$ ,  $X_2/X_7$ ,  $X_3/X_6$ ,  $X_4/X_5$ ,  $X'_1/X'_8$ ,  $X'_2/X'_7$ ,  $X'_3/X'_6$ , and  $X'_4/X'_5$  represent carboxamide binding pairs which bind DNA base pairs selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA

20 base pair in the minor groove to be bound;

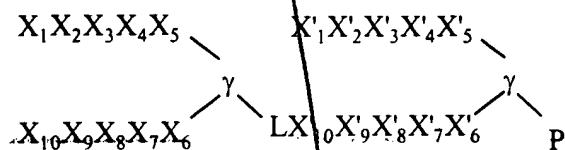
L represents an amino acid linking group selected from the group consisting of  $\beta$ -alanine and 5-aminovaleric acid ( $\delta$ );

P represents a polyamide selected from the group consisting of

$X_1X_2X_3\gamma X_4X_5X_6$ ,  $X_1X_2X_3X_4\gamma X_5X_6X_7X_8$ ,  $X_1X_2X_3X_4X_5\gamma X_6X_7X_8X_9X_{10}$ , and  $X_1X_2X_3X_4X_5X_6\gamma X_7X_8X_9X_{10}X_{11}X_{12}$  where  $X_1-X_{12}$  are independently selected from the group consisting of  $\beta$ -alanine, pyrrole, hydroxypyrrrole and imidazole; and

A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

- 5 22. A tandem-linked polyamide having the formula:



*partial and*  
10 wherein  $\gamma$  is -NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CONH- hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

15  $X_1/X_{10}$ ,  $X_2/X_9$ ,  $X_3/X_8$ ,  $X_4/X_7$ ,  $X_5/X_6$ ,  $X'_1/X'_{10}$ ,  $X'_2/X'_9$ ,  $X'_3/X'_8$ ,  $X'_4/X'_7$ ,  $X'_5/X'_6$  represent carboxamide binding pairs which bind DNA base pairs selected from the group consisting of Hp/Py, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound;

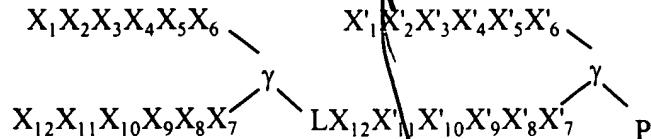
L represents an amino acid linking group selected from the group consisting of  $\beta$ -alanine, 5-aminovaleric acid ( $\delta$ ) and a derivative thereof;

20 P represents a polyamide selected from the group consisting of

$X_1 X_2 X_3 \gamma X_4 X_5 X_6$ ,  $X_1 X_2 X_3 X_4 \gamma X_5 X_6 X_7 X_8$ ,  $X_1 X_2 X_3 X_4 X_5 \gamma X_6 X_7 X_8 X_9 X_{10}$ , and  $X_1 X_2 X_3 X_4 X_5 X_6 \gamma X_7 X_8 X_9 X_{10} X_{11} X_{12}$  where  $X_1-X_{12}$  are independently selected from the group consisting of  $\beta$ -alanine, pyrrole, hydroxypyrrrole and imidazole; and

A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

23. A tandem-linked polyamide of claim 1 having the formula:



wherein  $\gamma$  is  $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CONH}-$  hairpin linkage derived from  $\gamma$ -aminobutyric acid or a chiral hairpin linkage derived from R-2,4-diaminobutyric acid;

$X_1/X_{12}$ ,  $X_2/X_{11}$ ,  $X_3/X_{10}$ ,  $X_4/X_9$ ,  $X_5/X_8$ ,  $X_6/X_7$ ,  $X'_1/X'_{12}$ ,  $X'_2/X'_{11}$ ,  $X'_3/X'_{10}$ ,  $X'_4/X'_9$ ,  $X'_5/X'_8$  and  $X'_6/X'_7$ , represent carboxamide binding pairs which bind DNA base pairs selected from the group consisting of Hp/Hp, Py/Hp, Py/Im, Im/Py, and Py/Py to correspond to the DNA base pair in the minor groove to be bound;

L represents an amino acid linking group selected from the group consisting of  $\beta$ -alanine, 5-aminovaleric acid ( $\delta$ ) and a derivative thereof;

P represents a polyamide selected from the group consisting of

$X_1X_2X_3\gamma X_4X_5X_6$ ,  $X_1X_2X_3X_4\gamma X_5X_6X_7X_8$ ,  $X_1X_2X_3X_4X_5\gamma X_6X_7X_8X_9X_{10}$ , and  $X_1X_2X_3X_4X_5X_6\gamma X_7X_8X_9X_{10}X_{11}X_{12}$  where  $X_1-X_{12}$  are independently selected from

the group consisting of  $\beta$ -alanine, pyrrole, hydroxypyrrrole and imidazole; and

A represents a positive patch consisting of a rigid group adjacent to a positively charged group such that a positive charge is delivered to the phosphate backbone or major groove of a DNA molecule.

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24. A polyamide of claim 1 selected the group consisting of:

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -RPR;

ImImPyPy- $\gamma$ -ImPyPyPy- $\beta$ -RPR;

5 ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -RP<sub>n</sub>RRR;

ImImPyPy- $\gamma$ -ImPyPyPy- $\beta$ -RP<sub>n</sub>RRR;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -R;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -RP;

10 ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -RGR;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -R<sup>D</sup>PR;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -APR;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -KPR;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -RPK;

ImPyPyPy- $\gamma$ -PyPyPyPy- $\beta$ -C7-RPR; and

15 the pharmaceutically acceptable salts thereof.

25. A method of inhibiting gene expression comprising contacting a regulatory sequence of a gene with a polyamide of claim 1.

20 26. A method of inhibiting gene expression comprising contacting a DNA molecule with a polyamide of claim 1 whereby the DNA molecule is conformationally constrained.